MANUAL
FOR THE DETERMINATION OF
SEED-BORNE DISEASES

EDITED BY
THE INTERNATIONAL SEED TESTING ASSOCIATION

Collected in collaboration with the members of the „Committee for
the determination of seed-borne diseases"
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PREFACE

This work has been accomplished in collaboration with the members of the „Committee for the determination of seed borne diseases" as may be seen from the heading of this publication.

I am highly indebted to the „International Seed Testing Association", which made it possible from a financial point of view to publish this manual. I also wish to express my warmest thanks to Dr. W. J. Franck for his idea of charging me with the composition of this edition of the I.S.T.A., for his suggestions and assistance in the composition of this manual and for the readiness with which he gave the opportunity to make the drawings in the laboratory as far as the official testing work permitted. Some special words of sincere thanks and appreciation to my collaborators Miss M. J. C. Schokker and Ir. Leendertz, who in such an able and artistic way enhanced the instructive value of this publication.

In this place I wish also to thank Prof. Dr. Joh[a]. Westerdijk and her staff most heartily for the kindness, with which they have always helped me when difficulties arose in the identification of several fungi. Further I am much obliged to one of the committee members Director J. Juhans as well as to Dr. E. Rogenhofer, who both sent some microscopic drawings and photographs for this illustrated manual, with the descriptions bearing upon them, and to the members Dr. E. Napravil, Prof. M. Kondo and W. F. Crosier, who added several valuable contributions to the list of seed infections. Several other details, referring to the testing of the seed with regard to its sanitary condition, have been taken from publications of the members Prof. G. Gentner, Prof. M. T. Munn, and others. The accompanying list of literature only contains those publications to which references are made in the text.

Though this manual cannot in any way be considered to be complete, it seemed desirable that its publication should not be longer delayed. It is to be hoped, that in the next few years many more

illustrated descriptions of seed-borne infections will be added to these, which are published now.

Finally I want to thank heartily Mr. T. Anderson and Dr. Mary Noble for the critical revision of the English text.

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GENERAL PART

GENERAL CLASSIFICATION

Investigation of the sanitary condition of seed must be based partly on the condition of the sample and the dry seed as sent to the seed testing station, and partly on the behaviour of the seeds during the germination tests.

When studying the condition of the sample and the dry seed there are the following possibilities:

I. The sample does not contain immediately recognisable carriers of diseases, symptoms of infections, or storehouse pests.

II. The sample does contain immediately recognisable carriers of disease, symptoms of infections, or storehouse pests.

   A. These can be macroscopically detected as:

      a. carriers of diseases intermingled with the seeds (bunt kernels, sclerotia, Tylenchus kernels, etc.);

      b. distinct spots, etc., on the seeds (Colletotrichum Lindemuthianum);

      c. damage caused by parasitic insects (Bruchus, Megastigmus, etc.);

      d. storehouse pests (granary weevils, etc.).

   B. They can be microscopically detected as:

      a. phases of fungus growth, distinguishable on the dry seed with the aid of a binocular microscope (Septoria petroselini, etc.);

      b. infection by fungus spores (Tilletia tritici, etc.).

When the behaviour of the seeds during the germination tests is studied, there are the following possibilities:

I*. The seeds produce apparently normal healthy seedlings,

   A. The seeds produce seedlings, which are actually healthy.

   B. The seedlings are affected by a disease, which cannot be detected by the methods hitherto adopted by the seed testing stations (smut of wheat, some bacterial infections, virus diseases, etc.).

II*. The seeds give rise to abnormal or diseased seedlings as a consequence of:
  A. damage caused by mechanical means, e.g. threshing, scarifying, etc. (broken seedlings).
  B. damage by insects, etc.
  C. decreased vitality often accompanied by the growth of saprophytic fungi or saprophytic bacteria.
  D. nutritive disturbances, resulting from soil conditions, (such as deficiency diseases like Marsh spot in peas).
  E. distinct diseases or infections
    a. caused by parasitic fungi, where we find:
      1. mycelium appearing more or less superficially in or on the seedcoat or on the pericarp as the case may be;
      2. mycelium penetrating more deeply.
    b. caused by parasitic bacteria;
    c. caused by insects living within the seeds (Bruchus, etc.).
III*. The seeds have not germinated at the end of the germination tests,
  A. The inside is healthy (seeds hard, or in a dormant state).
  B. The inside is dead and may be, as frequently happens, infected by saprophytic fungi or bacteria.
  C. The inside has been wholly or partly consumed;
    a. living insects can still be found inside the seeds (Megastigmus etc.);
    b. living insects are gone but the remains are still present, e.g. cocoons, excrements, etc. (Fritfly).

METHODS OF INVESTIGATION AND GENERAL REMARKS

II. A. In order to find out whether seed samples contain macroscopically easily recognisable carriers of diseases, symptoms of infection, or storehouse pests, a purity test of the seeds is required.
    a. It is necessary to determine whether there are carriers of diseases among the seeds, of which examples have been mentioned in the above general classification under II. A. a.
    b. Sometimes the dry seeds may show spots, which plainly indicate some sort of infection.
    c. The seeds themselves may also show symptoms of damage done by insects in the form of emergence holes, in which event there is a possibility, that in some of the rest of the

seeds these insects are still alive in a more or less advanced state of development.

  d. Storehouse pests like granary weevils, grain moths, mites, etc. too, can be discovered during the test.

II. B. Infections, which are microscopically at once clearly distinguishable should be further investigated with a binocular microscope or, if higher magnification is necessary, with an ordinary microscope.

  a. If phases of fungus growth, clearly visible on the dry seeds, have to be examined, this can be done in the following way: a certain number of seeds (50 or 100) are examined with the aid of a binocular enlarging about 25 times, to find out whether infection is present. The result of this determination can then be expressed as a percentage. If infection is present in the form of pycnidia, it is advisable to soak the seeds in water before they are examined as the pycnidia will then swell and can be more easily distinguished.

  b. Infection can also be present in the form of fungus spores, adhering to the outside of the seeds. The period of the germination test is not long enough for some kinds of spores to grow and develop characteristically, so that it is not always possible to detect such an infection during the germination tests. To find such an infection by adhering spores, one can shake a certain number of seeds with water, alcohol or methylated spirits in a test tube; afterwards on pouring out the liquid, one can find out with the help of a microscope enlarging 100 times or more whether bunt-spores or other spores are present. Before beginning this microscopical examination it is desirable, that the spores, present in the liquid, are concentrated in some way or other. This can be done in the following ways:

   1. by straining the liquid through filter paper, which is of such a texture, that the spores cannot pass through but remain on the paper (Gentner method [19]);

   2. by partly evaporating the liquid, which for this purpose is poured into a porcelain saucer and heated, shaking it from time to time, until any spores present are in a more concentrated suspension;

   3. by centrifuging the poured-out liquid in a test tube so that the spores, which are heavier than the liquid, are

thrown down rapidly. The clear liquid from the top of the settled spores can then be carefully decanted or removed with a pipette.

If, during a microscopical examination in this way of wheat, bunt spores are found, there is the question whether these Tilletia spores are still alive or not; the bunt spores may be dead because the wheat may have been already treated. A viability test of these bunt spores would make it clear whether they were really still alive, but such an examination, as part of the ordinary technique of seed testing, would certainly take too much time. In the above mentioned investigations, it is therefore only determined whether bunt spores are present, while the question of whether the spores have lost their vitality or not is left unanswered.

The above mentioned methods of determination apply to the condition of the seed as sent to the seed testing station.

I*. II*. III*. It is also of great importance to observe how the seed behaves during the germination tests. In order to form an opinion about the sanitary condition of the seedlings, it is necessary to experiment by methods different from those generally used. The use of folded blotting paper for seed-beds has this disadvantage, that the seeds may roll towards each other, thus providing an opportunity for moulds to extend over the healthy, normal seedlings, and cause them to rot. There are fungi, such as Botrytis cinerea and Fusarium, that are extremely injurious, causing healthy seedlings to wilt in a very short time. Other fungi, such as Penicillium, Rhizopus, etc., do not attack the healthy seedlings so readily although their presence may cause some difficulty in the formation of an opinion about the sanitary condition of the seedlings. It is also very important for these investigations, that the fungi, which may be present have enough time to develop characteristically, so that eventually they can be identified. For this purpose the germinated seeds must not be taken from the beds on the day of the energy test, as is the custom in ordinary germination tests, but they must remain a little longer. This period of their retention under test depends on the kind of seed; different seeds require different periods to make it possible for diagnosis to be made of their sanitary condition. This question will be dealt with more fully in the special chapter. For the reasons mentioned above, it is necessary to give the seeds more space for germinating and to put them on a firm seed bed; the seedlings will then not infect each other

so easily during the tests and they will have more room to develop. For this purpose it has been found practical at the Government seed testing station at Wageningen to use flat zinc trays, 27 cm × 10 cm in size, with perforated bottoms on which damp blotting paper has been laid (Plate I). Further, to ensure, that the seeds stay in place, this blotting paper can be indented in a regular fashion, usually with 100 spaced indentations, one for each seed. If, when zinc trays are used, the seedlings show symptoms of injury, such as described by Crosier et al. [11 and 12]) for seedlings of tomato and crucifers, the germination test should be repeated in blotting paper. Such injury-symptoms, however, are probably associated with the use of galvanized zinc trays. In our experience these injury-symptoms rarely occur when pure-zinc trays, regularly cleaned by brushing, are used. The trays can either be covered or not. During the examination of beet seeds, it is better to leave the trays uncovered — as in the ordinary germination tests of beet seeds, — because these require a less damp atmosphere for normal germination. Except in this particular case the trays are covered as a rule. When testing larger seeds that do not adhere so easily, such as those of cereals, peas, beans, etc., another sheet of wet blotting paper may be used as a covering (Plate I 2). In this way the germination bed is kept in a sufficiently moist condition, which for these experiments is of particular importance, considering that the cereals and peas used in these special investigations have not been soaked before they were put into the beds. The soaking of beans beforehand will be dealt with in the special chapter. When examining the sanitary condition of other kinds of seeds, such as flax seed, small vegetable seeds and others, covering with wet blotting paper may have a certain disadvantage. When this cover is removed at the end of the test, the ungerminated as well as the germinated seeds may adhere so firmly to it, that the seedlings may get broken to an extent which makes it very difficult to obtain an idea of their sanitary condition. In such cases, it has been found practical to cover the germination trays with a glass plate. For this purpose two flat strips of glass are laid within the tray, one along each end, and firmly held in place by wads of blotting paper; on these strips of glass the plate is laid (Plate I 3). The germination bed can be kept sufficiently moist by pouring a little water under the plate from time to time. The further process of germination is, in this way, easy to follow. If different fungus-centres or mouldy seedlings occur during the tests, they can easily be recognised under these conditions. Sometimes the fungi can be identified

at once with the naked eye, either by their typical way of growing or by the appearance of certain symptoms of disease characteristic of a certain infection, as for example the infection of beans caused by Macrosporium, which shows a violet discolouration with yellow margin. To detect other infections it may be necessary however to use a binocular with an enlargement of about 25.

As to the temperature of the cupboards where these tests of the sanitary condition are carried out, the same rules are applied here as for the ordinary germination tests. For beet seed intermittent temperatures of 20° C and 30° C are required. For flax seed, peas, and beans the temperature ought to be 20° C, and for cereals, where there is a possibility, that they have not yet afterripened, the temperature ought to be 10° C for the first three days and afterwards 20° C, until the day on which the germination capacity is determined. At the end of the test period, the length of which varies for the different seeds, it will in general be possible to notice:

I*. Apparently normal and healthy seedlings.

    Normal and healthy seedlings have full-grown roots, with well-developed root-hairs, while hypocotyl, cotyledons, and plumule also show that normal development and colour, which is characteristic of each kind of seed.

However, it may happen that:

    A. The seedlings are really healthy.

    B. The seedlings are actually carriers of infection, such as smut, bacterial diseases or virus diseases, which are difficult to identify and which cannot be recognised by the ordinary examination methods in use at the seed testing stations. As regards smut infection, this might be indicated by reference to a previous field inspection when required.

    When bacterial diseases cause no visible symptoms on the germinating seeds, it is still possible to detect the infection by an accurate microscopical examination. As a rule, however, such an examination takes too much time and can therefore not be considered as an ordinary method of testing.

    As to virus diseases, it is known, that in some instances they are carried in and perpetuated by seeds. In how far it would be possible to find out these diseases during the germination tests cannot yet be said. In this respect sowing tests have perhaps a better chance of furnishing an information, in which

case attention should be paid to curling of the leaves and the appearance of leaf blotch.

II*. During the germination tests abnormal seedlings or seedlings showing symptoms of disease can also be observed.

A. If during the tests the seedlings develop abnormally in consequence of breakages (broken seedlings), this fact is taken into consideration as part of the ordinary germination test, so that for this condition reference may be made to the rules for germination tests in general.

B. In instances of seeds being damaged by insects and other animals (storehouse pests, vegetation pests, etc.), which have not already been removed during the seed purity test, it is necessary to state whether the damage is really of such a degree, that no further growth can be expected.

C. Decreased vitality may result in stunted growth of the seedlings. The seed coat then has often lost its resistance against saprophytic bacteria and fungi, so that softening may occur as a result of decomposition caused by bacteria or infection by fungi such as Penicillium, Rhizopus and others. It is necessary to note the abnormalities for each kind of seed, so that they can be examined in each instance. The Cruciferae are already being observed with this object in view. In some cases the abnormality can be very typical. There may be mentioned here, as an example, the abnormal development where the tip of the root has died or is lacking. The appearance of this symptom, which may be considered as an indication of declining vitality, is a typical phenomenon of germinating lettuce seeds [33]) and also of germinating onion seeds [9]).

D. Another kind of abnormality is the result of soil defects, for example the presence of marsh spot in peas. With regard to this condition, it is necessary not only to observe the percentage of spotted peas, but also to note the degree of its severity.

E. During the germination tests particular diseases or infections can further be detected.

a. If parasitic fungous infections are detected, it is important to notice whether they

1. remain superficial, or
2. penetrate more deeply.

By cutting the seeds, by observing the cotyledons, etc., indications of a more or less deep penetration may often

be obtained. In a special chapter several of these fungous infections will be more fully dealt with.

b. Seeds infected by parasitic bacteria sometimes show discoloured spots caused by a concentration of bacteria under the seed coat. Such typical discoloration of the seed coat may be sometimes readily seen on the dry seeds, but often becomes clearer after the seeds have been for a few days in the germination beds.

c. There is also the possibility of the occurrence of insects within the seeds. Such seed infestations are chiefly caused by weevils, Chalcididae or gall-flies. These insects feed, when in the larval state, on the seed contents; then they pupate and finally leave the seeds as fullgrown insects. As a rule the eggs are laid in the ovules and develop within them. In that event the larva is not able to penetrate into ripe seeds. Such insects only have one generation each year and therefore the infestation does not increase during storage. Not so frequent is the case of those insects having several generations in one year, the larva then being able to penetrate into the ripe seeds. The result is, that such infestation may increase a great deal in storage, specially when the temperature is high. One way to limit the infestation is to maintain a low temperature; direct control may be obtained by means of insecticides. At the end of the tests it is necessary to determine by dissection whether there are still larvae, pupae, or adults in the seeds. It is further of importance to notice, when this proves to be the case, whether the insects are alive or dead.

III*. At the end of the tests a part of the seeds may still be ungerminated.

A. The question of these ungerminated seeds, which are hard is dealt with in that part of the general rules dealing with hard-seededness. If the ungerminated seeds are dormant, prolongation of the germination test or the application of a lower temperature would perhaps be more successful in bringing about germination. Neither does this case, however, fall under the heading of seed-borne diseases investigations.

B. If the seeds cannot be induced to germinate in consequence of lack of vitality, their substance has, as a rule, become more or less decayed through the activity of saprophytic bacteria

or fungi, while their exterior has generally also become mouldy on account of these organisms.

C. Finally, if the substance of the ungerminated seed has been wholly or partly consumed by infesting insects etc., there is
  a. a possibility that inside there are still living insects, which can be detected by cutting the seeds.
  b. another possibility that, though the inside of the seeds has been wholly or partly consumed, living insects are never found since they have already emerged; the presence of special cocoons, excrements, etc. may prove, however, that a certain animal infestation is in fact the cause of the seed's non-germination.

The above considerations are important because of their bearing on the possibility of improving the sanitary condition of the seed.

Finally, those saprophytic fungi, which very often occur in germination tests, should be mentioned. These fungi are not very selective, often growing abundantly on the moist filter paper of the germination beds or on the unglazed porcelain dishes. They can often be troublesome when appearing in masses. As such may be mentioned among others: Botrytis crystallina (Bon.) Sacc., Acremoniella atra Sacc. (Syn. Eidamia acremonioides (Harz.) Lindau), Papulaspora rubida Hotson, Papulaspora pisicola v. Beyma, Oedocephalum glomerulosum (Bull.), Acrostalagmus cinnabarinus Corda, Trichothecium (Cephalothecium) roseum Link, a.o.

IMPROVING THE SANITARY CONDITION OF THE SEED BY CLEANING OR BY TREATING

The great importance of testing the sanitary condition of the seed is not only, that certain seed-borne infections can be determined, but also, that when these infections are found in time they can often be controlled by some kind of treatment of the seed.

For instance, when sclerotia have been found in a sample, repeated cleaning of the seed may sometimes effect improvement.

This also applies to stocks of oats badly damaged by fritfly, since the injured kernels have a less specific weight than normal ones. In cases where fungous infections have been detected, disinfection can bring about a great improvement. A distinction must be made here, however, between bacterial and fungous infections, which are superficial and those, which penetrate more deeply. In general,

superficial infections, which occur more frequently than the deeper penetrating ones can be very well controlled by treatment. In testing the sanitary condition of the seed it is therefore often desirable to make disinfection tests. It is evident, that such laboratory tests can only be considered as preliminary and that the results should be corroborated by more extensive tests in the field. Field tests for the efficacy of disinfection are outside the domain of routine seed testing. This research work on disinfection can be done by making several germination tests simultaneously, with disinfected as well as with undisinfected seed, when it is important to observe whether the infection is sufficiently controlled by treating and whether the germination power does not suffer by treatment. Further it is advisable to have comparative sowing tests with disinfected and undisinfected seeds in soil; the favourable influence of treatment will result very often in a higher percentage of growing power. As to these disinfection tests in the laboratory, the following facts briefly can be noted: Dry disinfectants (dusts) or liquid disinfectants can be used. The first are used in powder form, being well mixed with the seed in the prescribed proportion. Sometimes the quantity of seed which one has at one's disposal for a disinfection test is so small, that it is impossible to weigh out the dust necessary for this purpose. In that event one can put the counted seeds (e.g. 100) in a test-tube and mix them well with a small quantity of disinfectant dust, afterwards shaking the treated seeds on a sieve in order to remove the surplus of disinfecting material. For accurate tests it is better to keep to the prescribed proportion. For wet disinfection a counted quantity of seed can be immersed for half an hour or longer in a solution of a prescribed concentration; afterwards the liquid is poured off and the seed dried again before being placed in the germination beds. This is the so-called immersion method. In practice the „Kurz-Beiz" method (short-time treating method) is very much used, in which a stronger concentration is used but less in volume in proportion to the weight of the seed to be disinfected. Thus the seed becomes less moist and can be dried more quickly. When the seed has to be sown not too long after having been treated, drying is then altogether unnecessary. This is not the place to detail the various materials of disinfection or disinfection methods. Every country has its own disinfectants, which are in use there and the necessary directions for use and explanations are commonly available.

Disinfection can only cause an improvement in the sanitary condition

when the infections have not penetrated too deeply into the seeds. If, on the other hand, an infection has penetrated deeply, the parcel of seed cannot be sufficiently improved and, if the percentage of infected seeds is high, must be disqualified for sowing purposes. A deeply penetrating infection, which is an exception to this rule and has a chance of being controlled by a hot water treatment is the smut infection of wheat and barley. There have been many publications on this hot water treatment in the last few years and therefore only a few of these will be referred to here. [16, 17, 18 and 28]

If the seed has been infested by insects, which are found still in a living state, the infestation can be controlled by applying insecticides or by heating the seeds until a temperature has been reached at which the insects die, the seeds themselves remaining undamaged.

SPECIAL PART

After the preceding general remarks, several abnormalities and infections must be more fully dealt with.

In this special part only a restricted number of seed infections will be discussed; others are only mentioned in the list which can be found at the end of this manual.

For some species of seeds, however, several infections will be discussed in such a way that the methods of testing the sanitary condition of seed in general will become sufficiently clear to include those infections which are not specially dealt with.

Where coloured pictures or drawings, relating to the infections discussed, have been made, a reference to them will be found in the text.

INFECTIONS AND INFESTATIONS CAUSED BY PARASITIC ORGANISMS AND STOREHOUSE PESTS IN:

CEREALS

In respect of cereals, attention will be given first to some infections, occurring in rye and also in wheat, oats, and barley, after which the typical infections for each of these kinds of cereals will be mentioned. F u s a r i u m infection is very important in cereals, because it may cause much damage subsequently in the field. By treating the seed, however, this infection can be controlled for the greater part. This disease can appear as secondary or primary infection, according to the definition given by Schaffnit. [31]) In the case of secondary infection the kernels have been infected during the last stage of ripening, that is in their last stage of development; in this period the fungus does not penetrate deeply and as a rule does not injure the germination capacity, so that it can be combated successfully by treating. In the case

of primary infection the kernel has become infected during the period between the flowering stage and the beginning of ripening and therefore the fungus does much more harm. The germination capacity, especially, often suffers considerably from such primary infection and is often completely lost. In accordance with the classification given above, two types of Fusarium infection must therefore be distinguished, namely, the secondary infection, that remains more or less superficial and the primary infection, where the fungus penetrates more or less deeply into the kernel with the consequence, that it can be controlled little or not at all by the treating of the seed.

Though the terms „primary" and „secondary" infection were originally used by Schaffnit with regard to infection by Fusarium nivale, they will probably also be suitable for those caused by other species of Fusarium. The primarily infected kernels may be detected in the germination beds after a lapse of some days by the development of sporodochia containing well-developed Fusarium spores. Those spores are, as a rule, more or less curved and multicellular by the occurrence of several septa. In this stage it is often possible to identify the exact species. The most frequent species affecting cereals are the following: Fusarium culmorum (W. Sm.) Sacc. with a violet mycelium and brown sporodochia containing rather broad Fusarium spores (Plate II); Fusarium nivale (Fr.) Ces. with small four celled spores; Fusarium avenaceum (Fr.) Sacc. (Plate III) and Fusarium herbarum (Cda.) Fr. with more slender and longer spores. For accurate identification of the different species, however, one should naturally consult the special literature on this subject [3, 35 and 36].

Along with these Fusarium spores or conidia it may happen, that perithecia, the ascigerous stage, are also formed. These perithecia contain asci, each having 8 ascospores of the same type as the above mentioned Fusarium conidia but smaller and as a rule provided with only three septa, in which case it appears often to be Gibberella Saubinetii (Mont.) Sacc., which has caused the infection and which is characterised by blue coloured perithecia.

A slight, secondary Fusarium infection of the kernels may be recognised during the germination tests by the appearance of mycelium on the rootlets and at the base of the developing seedlings, which, in consequence of this, are more or less brown coloured (Plate IV). Intermixed with this mycelium, small one-celled spores, microconidia, may be found. These microconidia are not characteristic for different Fusarium species, so that it is quite impossible to base an identific-

ation on such spores. Only in pure cultures, where under favourable conditions, macroconidia, the typical Fusarium spores, are found, is it possible to identify the true species.

The method, which is used as a rule at Wageningen for testing this Fusarium infection, is as follows:

the germinating cereal grains are inspected on the 7th day after having been put into the germination beds, for which purpose the flat zinc trays, with moist filter paper at the bottom, are used, such as has been described in the general part of the text. The percentage of brown discoloured rootlets is then fixed approximately and indicated as follows:

no brown, discoloured rootlets:			free from Fusarium
< 5% brown, discoloured rootlets:			very slightly infected by Fusarium
5–15% ,,	,,	,,	slightly infected by Fusarium
15–25% ,,	,,	,,	moderately infected by Fusarium
25–35% ,,	,,	,,	ratherheavily infected by Fusarium
35–50% ,,	,,	,,	heavily infected by Fusarium
> 50% ,,	,,	,,	very heavily infected by Fusarium

This Fusarium infection may also be tested by the Hiltner method [21]. By this method the cereal kernels are sown in ground brickstone, about 3 cm under the surface (Plate V), so that this test may be considered as a sowing test under unfavourable conditions. In this way the Fusarium infected seedlings can easily be recognised; one is not only able to state the approximate percentage of the infection but also its intensity. The heavily infected kernels yield spirally curved seedlings which are not able to pierce through the layer of ground brickstone. The less-heavily infected kernels grow into straight seedlings which, however, show a more or less brown discoloured base as a symptom of this infection. The scale on which the Fusarium infection is indicated in Munich according to this method is as follows:

Fusarium infection	1– 2%	. . . .	hardly any
,,	,,	2– 5%	. . . .	a little
,,	,,	5–10%	. . . .	slight
,,	,,	10–20%	. . . .	moderate
,,	,,	20–30%	. . . .	rather pronounced
,,	,,	30–50%	. . . .	heavy
,,	,,	> 50%	. . . .	very heavy

A disadvantage of this method is, that in accordance with the given directions the test is not finished before 14 days. At a time when many cereal samples are sent in for testing, however, quicker results of the tests are highly desirable.

The certifying of Fusarium infection in the germination beds by the method used in Wageningen can be made after 7 or 8 days, so that the results of this testing may be given at the same time as that of the ordinary germination test.

If, at the same time, parallel tests are made with treated seeds, the advantage of treatment will as a rule be seen very clearly.

Though Claviceps purpurea (Fr.) Tul. chiefly infects rye, sclerotia of this fungus may also be found exceptionally in wheat, oats, and barley samples, so this infection must also be dealt with as one of the general diseases, which may be found in cereals.

Should Claviceps sclerotia or ergots be found in cereal samples, they should undoubtedly be considered as noxious impurities, as they may give rise in the field to a renewed infection the next season. It is therefore desirable, that the sclerotia be removed by cleaning the seed. This can be done with good cleaning machines (e.g. oscillator). The large group of storehouse pests will now be dealt with. Though they are not confined to cereals, they may cause particularly extensive damage to this kind of seed and therefore they are mentioned in this connection. The storehouse pests are mostly insects. Especially if the temperature of storage is rather high, they may cause great damage; on the other hand, however, they are not parasites of the growing plants: in other words, the damage they do is confined to the seeds.

A well-known representative of this group is the granary weevil, Calandra granaria L., a brown-to-black coloured snout-beetle. The female bores a small hole in the grain with the snout, generally where the kernel is weakest, i.e. above the germ, and she deposits an egg in this opening. The larva feeds upon the contents of the kernel, pupates within it, and emerges at last a fullgrown beetle. As a female lays eggs during a long period and as four generations may develop annually at room temperature, it is obvious, that the harm caused by this beetle can become very considerable. For this reason the appearance of such beetles in cereal and other samples ought to be mentioned in certificates of seed analysis. Besides the granary weevil, other species of beetles and moths may also cause damage to the stores. To go further in the matter would take

up too much space. The reader is therefore referred to the list mentioned at the end and for further details to the standard work by Zacher [37 and 38]).

Together with the appearance of insects, attention should be paid to the possible presence of m i t e s in samples of cereals or other seeds. Especially if the degree of moisture of the bulk and the temperature are rather high, this infestation may increase a great deal during storage.

In addition to different species of Tyroglyphus and possibly also some other mite species, which live at the expense of the stores, predaceous mites belonging to the genus Cheyletus are often found. These mites prey upon flour-mites; the former, however, never succeed in thoroughly eliminating the latter. Both groups are balanced in a certain equilibrium, though the flour-mites remain as a rule predominant.

Where storehouse pests appear in a sample in a noticeable degree, this ought to be reported to the sender, so that he can destroy them by means of insecticides, such as carbon disulphide, Areginal, etc. or reduce the infestation as much as possible by means of low temperatures or by repeatedly turning over the seed and drying it as well as possible.

WHEAT, Triticum vulgare Aschrs. & Gr.

Besides those fungi already referred to as affecting cereals in general, bunt, Tilletia tritici (Bjerkander) Winter or Tilletia levis Kühn, may be found as an infection of wheat.

When testing for this infection, attention should be paid first of all to the possible appearance of bunt kernels (Plate VI), whether intact or broken. If these are not apparent, the wheat may nevertheless be infected by the load of bunt spores, which may be especially congregated between the hairs at the apex. As has already been said in the general remarks, it is possible to test wheat for the occurrence of such bunt spores (Plate VI) by shaking well a standard weight of kernels with some liquid or other, which is examined for the presence of spores with the aid of a microscope. This testing can be carried out by following the method of Gentner [19]), which has already been mentioned in the general discussion of the methods of testing. This is done in the following way: 10 gr of wheat kernels are thoroughly shaken with 15 ccm. alcohol or methylated spirits in a test-tube. This liquid is poured into a little copper cylinder with perforated bottom

on which a round filter paper is fitted. The bunt spores will remain behind on the filter paper. After shaking and straining off once more, the piece of filter paper is laid on a slide and made transparent by means of Xylol or Toluol; it is then microscopically examined for the presence of bunt spores. The spores occurring in the visual field are counted; this enumeration must be repeated in 10 different fields. The average number (Y) is accepted as the number of spores present per visual field. If the radius (r) of the visual field belonging to the applied enlargement and the radius (R) of the small round filter-paper are measured, the following formula gives the number of spores (X), which are really present on the paper:

$$X = \frac{R^2}{r^2} \times Y$$

Though the detection of the bunt spores between the fibres of the filter paper requires some practice, this method of testing can yet be well applied, as it gives quantitative results, and its application does not require too much time. Dounine and Michailow [14] tried by making some alterations in the operation of this method to obtain results, which are even more exact.

Bredemann [7] also worked out a method for determining the infection with bunt spores. As the kernels have to be ground up for this purpose, in accordance with the given prescription, this method seems particularly suited for testing flour, fodder, etc. for the presence of bunt spores.

Up till now, at the seed testing station at Wageningen, a method has been followed, which gives more comparable than accurately quantitative results. It is as follows:

100 kernels are flooded in a test tube with some hot water in order to drive away the air between the hairs at the apex and to loosen the spores, which may be adhering between them. After the water has cooled down sufficiently, the test tube is shaken well for about one minute, after which the water is poured into an evapourating dish. The shaking is repeated again, this time with some cold water, which is poured out and added to the first lot. This water is vaporised upon a hot-water bath until a small quantity of about $\frac{1}{2}$ ccm. remains. Some drops of this are put on a slide and by means of a microscope the bunt spores, which happen to be present are counted. If no spores are found, it may be said that the sample is free from bunt spores, as by this method of testing the very slightest infection can be detect-

ed. It may occur, that in testing several wheat samples only a few spores or some hundreds of spores are found. Consequently, by this method of testing, only comparable and not exact quantitative values for the rate of infection are obtained.

Besides bunt kernels, ear-cockles may be found in admixture with the normal grains. These are of the nature of galls, caused by a species of eelworm, Tylenchus scandens Schneid. When these kernels are sown with the others, the eelworms, which keep alive for several years, are able to spread the infection in the soil. The galls can be recognised by their dark brown colour; they have a thick coat and are filled up with a yellow soft mass, formed by an accumulation of eelworms. The supposition, that these eelworms, moreover, can transmit a second infection, the Dilophospora disease, has been expressed by Atanasoff [4]). This fungus infects the ears, colouring the diseased parts black.

A wheat sample can further contain kernels showing a dark colouring above the germ, which symptom is called the "blackpoint" disease [15]). This symptom often is caused by Helminthosporium sativum P. K. & B. (Plate VII), a fungus with dark many-celled spores. The infection remains as a rule superficial and therefore can be controlled by treating the seed.

OATS, Avena sativa L.

Amongst normal oat kernels badly damaged ones may be found; as a rule an empty cocoon of the fritfly (Oscinis frit L.) is found within the remainder of the kernels between the glumes. The second of the three generations, which the fritfly has in one season, lives at the expense of the young oat kernels. As a rule such kernels are so badly damaged, that germination is quite out of the question. The empty cocoon, about 2 mm. in size, from which the adult has, as a rule, already emerged in the field, is found between the glumes among the remains of the kernel. Should the fly still be found in the cocoon, as indeed sometimes happens, it is always dead. There can be no question of transmission of the infestation by seed containing living insects. As has already been mentioned in the general chapter, the damaged kernels are lighter than the normally filled ones, so that there is a possibility that the seed can be improved and its germination capacity raised considerably by repeated cleaning.

If the fritfly infestation is at all extensive, it is therefore desirable to mention it in the certificate of analysis so that the attention of the sender is drawn to the advantage of repeated cleaning with a view to the improvement of the quality of the bulk.

A fungus, which can be transmitted by the seed is Helminthosporium avenae Eidam, which causes a leafspot disease in the field. This seed infection can be detected during the germination tests by means of a binocular microscope after about 8 days, when one must look for the dark coloured multicellular Helminthosporium spores and their black, bristly conidiophores very much like Helm. gramineum (Plate VIII). Black oats especially may often show a heavy Helminthosporium infection. This infection may also be recognised when testing the seed by means of the Hiltner method [21]), that is by sowing it under a layer of coarsely-ground brickstone. After some 12 days peculiar spirally twisted seedlings have developed. The difference between these and the well-known types of the spirally twisted seedlings from Fusarium attack is, that the latter are highly twisted from the bottom onwards, whereas the winding of the Helminthosporium diseased oat seedlings begins a little higher. It is also characteristic of the winding, that the concave side is discoloured black and contains the fungus, which in all probability delays the growth and thus causes the contortion. By the germination, as well as by the Hiltner tests, it becomes evident, that this Helminthosporium infection can very well be controlled by treatment of the seed.

Until recently it was doubtful whether the smut infection of oats, caused by Ustilago avenae (Pers.) Jensen, could always be determined with certainty. Researches made by Zade [39]) seemed to reveal, that the greater part of the wind-borne smut spores, which lodge between the glumes, immediately begin to germinate, penetrating thus into the glumes and forming there a resting mycelium. If, therefore, on shaking oat grains in water, in the manner described for the determination of Tilletia-infection in wheat, one finds no Ustilago spores, it still remains quite uncertain, whether the infection is nevertheless present in the form of mycelium, as the detection of this latter kind of infection presents difficulty. According to a recent publication by Kitunen [24]), however, it is most unlikely, that the Ustilago spores are capable of forming such a wintering mycelium. Kitunen only found the Ustilago spores forming sporidia, which latter, moreover, soon lost, in dry condition, their germination power. In accordance with this finding therefore, the smut spores

should still, in the meantime, be considered as the sole wintering form of oat smut.

RYE, Secale cereale L.

In addition to Fusarium infection and that caused by sclerotia of Claviceps purpurea, which are both important, especially for rye, another infection, caused by spores of Urocystis occulta (Wal.) Rab. may be found. This fungus causes the smut disease of rye. The spore load can be detected and estimated by shaking the kernels with water and then carrying out the test in the same way as has been described before for Tilletia infection of wheat. The Urocystis spores are characteristically formed, being, as it were, compound spore-balls, with dark spores in the middle and slightly coloured, sterile spores around them.

BARLEY, Hordeum vulgare L.

Barley may be infected by Fusarium and Claviceps as well as by Helminthosporium. Three of these Helminthosporium species are of much importance for barley. The most noxious is Helminthosporium gramineum Rab. causing the stripe disease. The two others cause leafspot diseases: Helminthosporium teres Sacc. causes the so-called net-blotch disease, Helminthosporium sativum P. K. & B. the spot-blotch disease [15]). If barley kernels remain in moist germination beds for about 7 days, it is often possible to find Helminthosporium conidia and conidiophores, which have developed on the kernels (Plate VIII).
For their detection the use of a strong magnifying glass or, better still, of a binocular microscope is required. It has been, however, impossible up till now to distinguish in the germination beds the two species, Helminthosporium gramineum and Helminthosporium teres, by the form of their conidia and conidiophores. The top of the conidiophore bears one, sometimes two, spores, while a little under the top one or more lateral spores may appear at different heights. These spores are greyish-green, many-celled with transverse septa, not wider in the middle than on the rounded ends and not curved but straight.
Helminthosporium sativum is characterised by conidiophores, bearing at the top sometimes two, but as a rule, 4 or 5 spores; these are divid-

ed by transverse septa, and, when examined under the binocular microscope, are shining black. They are curved and widest in the middle (Plate IX 2). If this type is found on the kernels, it is to be expected, that the spot-blotch disease will appear afterwards in the field. If, on the other hand, spores of Helminthosporium gramineum or Helm. teres are found in the germination beds, one sometimes succeeds by means of a sowing test after the Hiltner method in determining which of the two diseases, the stripe- or the net-blotch disease, will appear in consequence of this infection. If, when the seedlings are examined, some are found with long brown stripes along the stems, it can then be stated with certainty that the seedlings have been infected by Helminthosporium gramineum.

The occurrence of these different Helminthosporium species on samples of barley makes it more or less possible, for Holland, to base a conclusion about the origin of the sample. Whereas Helminthosporium gramineum is not an uncommon feature of homegrown barley in this country, Helminthosporium sativum is seldom found. So, if a notable Helminthosporium sativum infection has been observed in a sample, it may be certain that the lot has been exported either from U.S.A. or from another exporting country.

As to covered smut of barley Ustilago hordei (Pers.) Kellerm. & Swingle, it may be assumed, that the spores of this species are windborne and deposited on healthy kernels in the field. The spores can be detected in the same way as has been described for Tilletia spores on wheat, only it is much more difficult to identify those small $(6-10\,\mu)$ brown Ustilago spores than the more typical Tilletia spores, especially if they appear only sporadically.

GRASS SEEDS

In samples of grass seed Claviceps sclerotia often occur. Should this be the case, they often belong to the species Claviceps microcephala Wallr., which forms stalked heads, that bear the sunken perithecia in the same way as Claviceps purpurea, but of much smaller sizes. Agrostis seed samples especially may contain them in a high degree.

Another example of damage, which may appear in grass seeds, e.g. Alopecurus, is caused by the larva of a gall-fly, Oligotrophus alopecuri E. Reut. The presence of the orange coloured pupa of this insect can be detected by transmitted light. As these pupae found

between the glumes are dead as a rule, this impurity need not be numbered among the noxious ones. It hardly ever happens, that samples of Alopecurus seed are absolutely free from them.

Samples of Trisetum flavescens P.B. sometimes contain dark coloured kernels, which are filled up with a white, cheesy mass. When examined under the microscope, this latter mass proves to be an accumulation of eelworms, Tylenchus trisetum Kühn (Plate X). Dr. Rogenhofer reports, that the seedproduction may suffer a great deal in consequence of this infestation.

Fusarium- and Helminthosporium-infection is also often found on grass seeds.

The bacterial disease of Dactylis glomerata L., caused by Erwinia Rathayi, is characterised by an exudate of yellow slime on the seed.

PEAS, Pisum spp.

In pea samples the damage caused by the larvae of the pea moth Grapholitha nebritana Tr. or Grapholitha dorsana F. can immediately be discerned. The larvae of these moths are found, when the plants are still in the field, inside the pods, where they injure the seeds irregularly and more or less deeply. Whether such seeds are still able to germinate, depends on the place and the extent of the damaged area. Even though the embryo has not been damaged, so that further growth is not out of the question, the resistance of such seeds against soil organisms has suffered a great deal. Such seeds are therefore of an inferior quality and are better removed from the bulk.

The pea weevil, Bruchus pisorum L., lives as a larva within the seeds, in which it pupates and from which it emerges through a round hole as a fullgrown weevil. In some rather warm countries this insect may become a real plague; any infestation of the stocks imported from such countries is, as a rule, proportionally very severe. When detecting this infestation, it is important to observe whether the insects, of which never more than one in a pea is found, are living or dead, as commercial seed infested by Bruchus has sometimes been previously disinfected with carbon disulphide. If, however, they are found still alive, it is desirable to recommend disinfection in the certificate of seed analysis. As the beetle remains alive for but one year, only dead insects are found in seeds, which have been stored for a longer time. The beetle Apion vorax Hbst. makes the same round holes in the

peas when emerging as Bruchus pisorum L. does, only, the diameter of the first is somewhat smaller.

Peas are tested for the occurrence of fungus infections, after having remained 5–6 days in the moist germination beds, as the fungi can develop and grow during this period.

Ascochyta pisi Lib., one of the fungi causing the spot-disease of the peas, usually grows at first as a white mycelium, in which the light brown pycnidia soon develop. Out of these pycnidia two-celled spores emerge in great masses, which are pink coloured (Plate XI). This infection can readily be determined in percentage with the naked eye. As this fungus can penetrate very deeply into the seeds, treatment has little or no perceptible result. Especially with horticultural pea-species, such as sugar-peas, heavy infections may occur. The agricultural species in general show less serious infections, though the infection can sometimes be of importance in these species also.

Besides Ascochyta pisi there are still two other Ascochyta species 23 and 34) which can be transmitted by the seed, namely Ascochyta pinodella Jones and Mycosphaerella pinodes (Berk. & Blox.) Stone. Ascochyta pinodella has smaller spores ($\pm$ 4.1 $\times$ $\pm$ 8.1 μ) than Ascochyta pisi ($\pm$ 4.9 $\times$ $\pm$ 15.8 μ) and so can immediately be identified by these spores. In Mycosphaerella pinodes the perithecial stage is known; for that reason it has been classed in the genus Mycosphaerella. A pycnidium stage resembling that of Ascochyta pisi also belongs to this species. A coloured illustration of this infection on the seeds can unfortunately not yet be supplied, as the true symptoms on the diseased seeds are not sufficiently known at present. It is probable, that this infection penetrates less deeply than that caused by Ascochyta pisi and thus can be better controlled by treatment. The infection by Macrosporium commune Rab. (Plate XVI 5), a fungus with dark, pluricelled spores, causes a violet discoloration surrounded by a yellowish margin on the seed coat of germinating peas. This infection, however, is less frequently found on peas than on beans. Infection caused by Fusarium spp., Botrytis cinerea Pers., Cladosporium herbarum Link., etc. can also occasionally be found on germinating peas. Peas with a lessened vitality often are invaded by saprophytic bacteria and in consequence grow mucilaginous during the germination tests (Plate XII).

Whether or not peas show the abnormality known as marsh spot must be tested by cutting the seeds in two. This can be done when they have been in the moist germination beds for some days. Peas,

which have this defect show a somewhat sunken brown area in the middle (Plate XIII). The cause of this abnormal condition has not yet been quite determined, but infection by bacteria or fungi is out of the question; influence of soil conditions should rather be considered. Researches made by Marie Löhnis [25]) and by Pethybridge [30]) make it appear probable, that these marsh spots are a result of a lack of manganese. In the Netherlands such abnormal peas occur especially in the polder districts. The damage, caused afterwards in the field by this abnormality depends on the greater or lesser extent of these brown areas. If, during the development of the seedling, the growing point of the plumule turns out to be brown coloured and dead, then the growth of the seedling is retarded. Even then, however, further growth is not completely out of question, as, in this case, the axillary buds of the cotyledons will develop. In consequence of this the seedlings need not necessarily die, even when they have been seriously damaged, but, when compared with healthy seedlings, they lag behind considerably in development. When, therefore, this marsh spot defect is detected in a sample of peas to some extent, it is necessary to indicate this condition on the certificate.

BEANS

DWARF- AND RUNNERBEANS, Phaseolus spp.

As before, those infections have to be considered first, which can immediately be identified on examining the dry seeds. This is the case with the infestation caused by Bruchidius obtectus Say (Plate XIV). The damage caused by this weevil is worse than the one caused by Bruchus pisorum in peas, as Bruchidius obtectus has more than one generation a year, and the larva moreover is also able to penetrate into the ripe seeds. Consequently, this infestation may increase very much during storage, especially if the temperature is rather high. The beans gradually become perforated with numerous round holes, out of which the full grown weevils have emerged. It is evident that a parcel of beans in this condition becomes absolutely worthless for sowing purposes as well as for consumption.
The beans can be tested for different fungous infections after having been laid for some 4 or 5 days in moist germination beds.
However, some remarks should first be made about the soaking of beans. It is well known that beans with diminished resisting power cannot stand soaking so well. If such beans, after having been soaked

for 5 hours, are put into the germination beds, after some 4 days they will be partly found mucilaginous without showing a trace of germination as a rule (Plate XVIII 1) whereas the unsoaked beans of the same sample may still show a rather high percentage of germinated seeds, which are not mucilaginous. This is chiefly due to the influence of bacteria against which fresh, viable beans are, to some extent, immune. As the influence of soaking on a certain sample of beans cannot be predicted, it is necessary to soak half the quantity of beans required for the germination test, while the other half is put unsoaked into the germination beds.

Of the fungous infections, which eventually appear the one caused by Colletotrichum Lindemuthianum Sacc. et Magn. (Plate XV) must be mentioned first. This fungus causes the well known spot disease of beans. This infection, if serious, may be already identified on the dry seeds, especially on beans with a light coloured seed coat, where it appears as grey-brownish spots. In order, however, to determine the exact percentage of this infection, it is necessary to put the seeds in a moist germination bed, because only then the less heavy infections can also be detected. Numerous pink coloured spores encircled by dark setae are readily formed in open spore cases (acervuli). It is much more difficult to recognise this infection in dry beans, which have a dark coloured seed coat. As this fungus often penetrates very deeply into the cotyledons, treatment of the seed is as a rule not very efficient; disinfection may be recommended only if the infection is slight and the fungus remains rather superficial. At all events the spotted beans should be removed by picking them from the bulk as well as possible. The Macrosporium infection of beans, caused by Macrosporium commune Rab. (= Pleospora herbarum (Pers.) Rab.) is often readily recognisable on the dry seeds by the appearance of a small pink-coloured spot round the micropyle (Plate XVI 1). This black spored fungus penetrates as a rule through the micropyle into the seed, usually not deeply, so that the infection can generally be very well controlled by treatment. If such beans are put into a moist germination bed for some days, this pink coloured spot becomes considerably enlarged and then becomes violet-red, surrounded by a yellowish margin (Plate XVI 2. 3). Multicellular dark Macrosporium spores (Plate XVI 4) are found sporadically between the grey mycelium, which will grow in this place. Often little black bodies develop on the infected seeds or on the filter paper a little above the seeds, representing another stage of development

of this fungus, namely the unripe perithecia, i.e. the ascus stage, connected with the conidial stage of Macrosporium. This ascigerous stage of the fungus belongs to the genus Pleospora. Macrosporium infection remains as a rule rather superficial, and treatment of a parcel of beans thus diseased often improves the sanitary condition a great deal. Dry disinfectants are preferable for treating beans, since wet dressing causes them to swell and, on drying again, to become wrinkled. The beans may also be infected by Fusarium spp. Such diseased seeds often germinate very badly, if at all. As a rule a white mycelium, with pink coloured sporodochia, grows out of these beans.

Moreover, beans can be infected by Botrytis cinerea Pers., which fungus will readily develop in its own typical way during germination. The infection by Cladosporium herbarum Link (Plate XVII 1 and 2) is, as a rule, only superficial and therefore hardly injures the germination capacity.

Among the parasitic bacteria, which may infect beans Bacterium flaccumfaciens Hedges must be mentioned. These bacteria can accumulate locally under the seed coat, where they cause a yellowish discoloration.

Another bacteral disease of beans, the halo-blight, is also transmitted by the seed. This disease is caused by Pseudomonas medicaginis f. sp. phaseolicola Burk.. Ivonne le Cosquino de Bussy [10]) recently made a study of this infection and succeeded in discovering bacteria in the epidermis of the seed coat. As such a test would require too much time for the ordinary testing work in the station, this infection could probably better be determined by means of sowing tests in soil. The diseased seedlings soon show a stunted growth, while the plumule is often discoloured brown and soon begins to wilt – vide the above mentioned publication.

Saprophytic bacteria often invade beans, which show a decreased vitality, making them soft and mucilaginous (Plate XVIII 1). This is particularly true of beans, which ripened and were harvested under unfavourable conditions and with those, which were harvested a few years before testing. In this case the seed coat more or less loses its immunity against micro-organisms, so that these find a suitable substratum here and cause decay of the seeds. The growth of these bacteria is often quickly followed by Penicillium infection (Plate XVIII 3 and 4). Such saprophytic bacteria are also the cause of the soaked beans becoming as a rule much more mucilaginous than unsoaked beans of the same sample. This condition therefore is an indication

of a great decrease in resistance. In consequence of this it may be anticipated, that the seed will have little strength to push through the soil, especially under unfavourable conditions, and that the percentage of strong-brairding seedlings will be much less than the germination capacity would give reasons to suppose.
In consequence of damage by threshing, broken seedlings may occur in the germination beds (Plate XVII 3).

BROAD- AND FIELDBEANS, Vicia faba L.

Though the dry seeds can immediately be tested for the presence of the weevil species, Bruchus rufimanus Boh. and Bruchus granarius L., it often happens, that during the germination tests a greater percentage of infested seeds are found, as a consequence of the presence of less developed stages of the insects, which are to be found within the seeds. Such stages can be detected by cutting the seeds in two at the end of the germination tests. This infestation is stationary, just like that of Bruchus pisorum of peas; it cannot increase during storage, because the larva can only penetrate into the young seed buds in the field. In contrast to the infestation of peas, however, more than one insect can be found at the same time in one seed in broadbeans. Sometimes an Ichneumonid, in some stage of development, can be found instead of the weevil, which thus has been parasitized.
As to fungous diseases, Fusarium sp. and Penicillium spp. may be found on these seeds in the germination beds. The beans may also become more or less mucilaginous in consequence of bacterial infection.

CLOVER, Trifolium spp.

Mixed with clover seeds one sometimes finds sclerotia, which may belong either to Sclerotinia trifoliorum Erikss., or to Typhula trifolii Rostr. The former are black and irregularly sulcate, the latter are round ($\pm$ 1 mm.) regularly sulcate and dark brown coloured (Plate XX 1). Sclerotinia trifoliorum, which causes stem rot of clover, may be transmitted by sclerotia, as has been described by Eriksson. Mrs. N. L. Alcock [1]) and M. S. Martin [2]) have described a fungus on white clover, resembling Sclerotinia trifoliorum Erikss. but which occurs as a mycelium on the seed and not, so far as is known, in the form of sclerotia mixed with seed. This mycelium occurs on the inside as well as the outside of the seed coat. Seeds thus affected may be

identified in the purity test by their lack of development, thin shrunken appearance and grey pink colour, while under a magnification of 16 to 20 times, flecks of mycelium may be seen on the testa. If infected seeds are kept in a damp atmosphere, small sclerotia are developed on them and such seeds usually fail to germinate. The only morphological point of difference between this fungus and Sclerotinia trifoliorum Erikss. is the small size of the apothecium, especially the disc, and of the sclerotium, so that at present the fungus is regarded by Alcock as a small form of Sclerotinia trifoliorum Erikss.

In a recently published study on Sclerotinia trifoliorum Erikss., however, Pape [29]) is of opinion, that the above mentioned clover seed fungus is not identical with the clover stem rot fungus, but is probably a new Sclerotinia species.

On seeds of Trifoliorum pratense L. in the germination beds, J. Juhans could detect an infection caused by Botrytis anthophila Bond. (Plate XIX). Besides the microscopic drawings illustrating this fungous infection, we also owe the following details to Juhans: Botrytis anthophila infects the stamen of red clover during flowering, as has been observed and described by Bondarzew [6]) and develops in the anthers between the pollen-grains. From there the seeds too become infected, while the fungus remains as a resting mycelium underneath the seed coat.

On clover seeds in the germination beds the following fungous infections may appear: Fusarium sp. (Plate XX 2), Macrosporium sarciniforme Cav., Botrytis cinerea Pers. and Botrytis trifolii v. Beyma. Treatment of clover seeds often results in a considerable improvement of the sample. The seeds of Medicago lupulina L. very often show infection caused by Phoma sp.

BEETS, Beta vulgaris L.

Phoma betae Frank., one of the fungi, which may be the cause of root rot of the young seedlings, is a very common seed-borne disease in beet seeds. Though this Phoma infection appears almost without exception in every lot of beet seeds, the damage, which it afterwards causes to the seedlings growing in the field, depends very much on the external circumstances, such as soil and weather conditions, especially during the first stages of growth. If these conditions are favourable for the seedlings, the fungus often does not cause much harm; if, however, the external circumstances are unfavourable,

many seedlings will perish in the field. The fungus can be detected on the seeds by examining them with a binocular after they have been in the germination beds for about a fortnight. Numerous Phoma pycnidia will then be found not only on the clusters but especially on the brown discoloured seedlings (Plate XXI). When determined in this way very bad infections may often be found, even up to 80% or 90%. One sample may, however, show as bad an infection as another and yet the first may produce seedlings, which are much more resistant to the infection than the second. Thus in the case of Phoma infection it is not only the percentage of infected seeds, that counts but also the greater resistance of the seedlings, for instance by more rapid development. Tests of the degree of resistance to this root rot fungus have been carried out in the Agricultural Laboratory of Prof. N. Ryoff at Moscow. It was proved by this investigation, that many slightly infected plantlets can recover completely. Although the degree of the Phoma infection in the germination beds has only a relative value, its determination is still of importance for the estimation of the effectiveness of various disinfectants. Beet seeds were treated several times with a Germisan solution or dusted with UT. 685, and the results are always a considerable decrease of the infection, often the infection disappears altogether after treatment.

Sometimes a test of beet seeds for the presence of rustspores, Uromyces betae (Pers.) Tul., is requested. This can be done by shaking the beet seeds in water and by carrying out the test in the same way as has been described for bunt infection of wheat. The Uromyces spores may be easily identified because of their relatively large size and typical rustspores-form.

FLAX, Linum usitatissimum L.

On flax seeds the following fungous infections can be detected on the day of the determination of the germination capacity, that is on the 7th day: Botrytis cinerea Pers., forma lini v. Beyma, and Colletotrichum linicolum P. et L. The Botrytis infection can be determined with the naked eye: the infected seedlings are then decaying, whilst the grey mycelium is developing (Plate XXII). As this fungus spreads rather quickly, it is often difficult, especially with samples, which have a high percentage of infection, when the zones of fungous growth run into each other, to indicate exactly from how many centres of diseased seedlings the fungous development has

taken place. It is, however, quite possible to give the degree of the infection in an approximate percentage. Disinfection tests are especially instructive here, as Botrytis infection can be controlled very well by treating the seed and as consequently the difference between parellel tests with treated and untreated seed can be very great. Flax seed can best be treated by means of dry-disinfectants, among which Ceresan dust gives excellent results.

In contrast to the naked-eye determination of the Botrytis infection, the use of a binocular microscope is indispensable for the determination of the degree of Colletotrichum infection. By an enlargement of about 25 times the pink coloured acervuli and the setae can be easily distinguished (Plate XXIII). This infection too, which afterwards causes ,,flaxcanker" in the field may be very well controlled by suitable dusting.

CABBAGE, Brassica spp.

Various investigations [8, 13 and 22]) have proved, that the fungus P h o m a L i n g a m (Tode) Desm., which is the cause of black leg of cabbage, is seed-borne; sometimes pycnidia of this fungus with exuding pink coloured spores are found in the germination beds on some seeds, especially on the ungerminated ones. Probably the brown stripes and spots, which are found rather often on the hypocotyl of those seedlings (Plate XXIV) in the germination beds are also caused by this Phoma infection. Whether this fungus is the only cause of the appearance of such stripes has not yet been stated with certainty; the brown spots on the cotyledons must probably often be attributed to infection by Alternaria sp. also. It is, however, a fact, that all these symptoms of disease are considerably decreased by treatment with solutions of mercury preparations or with dry-disinfectants. If a similar treatment is not wholly efficacious, the cabbage seeds may also be treated by means of a hot disinfectant solution or merely by a hot water treatment [13]). Brassica seeds may further be infected by various species of Alternaria, namely A l t e r n a r i a c i r c i n a n s (Berk. & Curt.) Bolle (Plate XXV 1), A l t e r n a r i a b r a s s i c a e (Berk.) Bolle (Plate XXV 2) and A l t e r n a r i a h e r c u l e a E. & M.

As Munn [26]) has observed and mentioned in a recent publication, several fungous infections are so typical of cabbage seeds of different origin, that he was able, by studying those fungi, to make conclusions about the provenance of several brassica samples.

CELERY, Apium graveolens L. and PARSLEY, Petroselinum sativum Hoffm.

A very common seed-borne infection of these seeds is the one, which causes the leaf spot disease of the adult plants, namely Septoria apii (Briosi & Cav.) Chester and Septoria petroselini Desm. These fungi are characterised by pycnidia, containing filiform conidia, which, as has previously been observed, are already present on the dry seeds. These pycnidia, which are found especially on the ribs of the seed (Plate XXVI) can easily be detected by means of a binocular microscope and better still when the seeds have been laid in water for a short time beforehand, causing the pycnidia to swell, and so rendering them easier to recognise.

Phoma pycnidia can also appear on celery seed during the germination tests. This infection has been studied by Goossens [20]) and it became clear from this research that, at least in the Netherlands, these seed-borne Phoma pycnidia do not belong as a rule to the parasitic species, Phoma apiicola Klebahn, which is the cause of the celery root rot. As a rule these seed-borne Phoma pycnidia will probably be of a saprophytic nature and as such of secondary importance for the sanitary condition of celery seeds.

Alternaria radicina M. & Dr. & E., was stated by Neergaard [27]) to be the cause of a serious infection of celery seed, doing much damage; as a consequence of this infection many seedlings perish in the field.

CARROT, Daucus carota L.

Carrot seeds are also often more or less badly infected by the above mentioned Alternaria radicina M. & Dr. & E. If such infected seeds are put to germinate in a germination bed covered with a glass plate as has been described in the general discussion of the methods, it is possible to observe on the day of the determination of germination capacity, that such seedlings have become black discoloured and decayed (Plate XXVII). This fungus, which is characterised by dark clavate multicellular spores, can also be the cause of the poor development or of the wilting of the seedlings in the field. This infection, as it remains superficial, can be very well controlled by treating the seed.

SPINACH, Spinacia oleracea L.

In the germination beds pycnidia of Phoma sp. may be found on the germinating seeds; these can be detected also on the rootlets of the seedlings, showing symptoms similar to those of Phoma infection in germinating beet seeds. Yet this infection does not appear so often on spinach seeds nor in such a high degree as is the case with beet seeds. Much damage in the germination of spinach seeds may be caused by another fungus, Colletotrichum spinaciae Ell. & Halst., which sometimes appears in masses. This fungus can easily be detected, when a binocular microscope is used, by the pink coloured acervuli set with dark setae (Plate XXVIII). Treatment of the seed is very effective in controlling both infections.

LETTUCE, Lactuca sativa L.

A rather unfavourable sanitary condition often occurring in germinating lettuce seed is not caused by a definite infection but by an abnormality, typical of this seed. During the germination tests seedlings often develop with abnormal roots, the tip of the root, which is brown, having died (Plate XXIX). Voisenat [33]) has thoroughly studied this abnormality and he succeeded in bringing it about artificially. By exposing fresh seeds to a high degree of humidity for some time and drying then off again, he obtained several seedlings which showed the typical brown root tip. It was apparent from this that the abnormality was not caused by a special infection, but, probably, by a check in the initial stages of germination. In soil tests it appears, that such seedlings are unable to continue growing. Sometimes such roots, with brown tips, give rise to a lateral root immediately above the dead part, which is able to take over the function of the main-root. In consequence of this, further growth is not absolutely excluded, but such plants are not of much practical value. As well as these typically brown root tips lettuce seedlings may show all kinds of brown spots on the cotyledons and on the hypocotyl which, when spreading, inhibit further development.
As a fungous infection of germinating lettuce seed Botrytis cinerea Pers. may be mentioned, which infection, as a rule, can be controlled by treatment. As lettuce seed sometimes is rather sensitive to disinfectants it is advisable to make a preliminary test each time treating of the lot seems necessary.

38

ONION, Allium spp.

An infection, which is probably only exceptionally transmitted by the seed is the onion smut, caused by Urocystis cepulae Frost. The wind may transmit these smut spores to seed harvested from plants which were originally healthy. Urocystis spores may be detected in the same way as Tilletia spores in wheat, that is to say, by shaking the seeds with water. If, by such a test, Urocystis spores are detected, the seed should certainly be disinfected. Often seeds are found in the germination beds on which unripe perithecia of Pleospora sp. occur together with its conidial stage, Macrosporium sp. Several Botrytis species may also appear on germinating onion seeds; one of them belongs to the species Sclerotinia porri v. Beyma [5]).
Among these onion seedlings abnormally formed ones may appear showing rootlets that end in an obtuse tip. In this case, however, the tip is never brown discoloured as was described for lettuce seedlings, but is altogether lacking (Plate XXIX). The obtuse end is sometimes yellowish discoloured in consequence of bacterial infection; Penicillium also often develops at this point. The researches of Grace Cole [9]) have shown that, as a rule, further growth is, in this case, out of the question.

BLACK SALSIFY, Scorzonera hispanica L.

It may happen, that mycelium is seen growing on Scorzonera seeds in the germination beds. The infection may appear as a white mycelium, in which the sclerotia of Sclerotinia sclerotiorum (Lib.) Massee are formed readily (Plate XXX 1). If, on the other hand, sclerotia develop after a grey mycelium with conidiophores and conidia has been formed, the infection has been caused by Botrytis cinerea Pers. Both fungi usually appear superficially, so that treatment of the seed may result in a great improvement.

TOMATO, Lycopersicum esculentum Mill.

Schoevers [32]) was able to state, that the tomato canker disease, caused by Didymella lycopersici Klebahn (= Diplodina lycopersici Hollos.), may be transmitted by the seed. He detected on seeds, which were collected purposely from diseased fruits, after he had left them in a moist condition for 4 to 7 days, pycnidia with two-celled spores, which are characteristic of Diplodina.

Pycnidia with one-celled spores, probably belonging to the genus Phoma, often appear on tomato seeds during the germination tests. With respect to these infections, the treatment of tomato seeds is often desirable. This has to be done, however, very carefully, as these seeds are rather sensitive to some disinfectants, the germination capacity being easily damaged by treatment. It is therefore advisable to make a preliminary test with a small quantity of seeds before deciding to treat the whole lot.

CORN-SALAD, Valerianella olitoria Poll.

These seeds are often more or less infected by Phoma sp. On the seedlings the pycnidia of this fungus can easily be detected by means of a binocular microscope. In soil tests it becomes evident, that this fungous infection causes much harm, but may, on the other hand, easily be controlled by treating the seed.

TREE SEEDS

After this discussion on various agricultural and horticultural seed infections, some of those, that occur on tree seeds should also be considered.
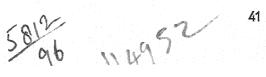
As a fungous infection of several conifer seeds (Thuya, Chamaecyparis, Cupressus, etc.) Pestalozzia sp. may be mentioned. This fungus is characterised by multicellular dark spores, which bear, on colourless end-cells, two or more hyaline cilia.

Chalcidides, a group of wasps, are found in several conifer seeds. The females of those insects are provided with an ovipositor, with which they lay the eggs within the young seeds. The larvae live in those seeds and feed upon their contents. They cut a round hole in the seed coat through which the adults emerge after pupating. The infected seeds have consequently lost their germination power entirely and they moreover spread the infestation as long as there are still living insects inside. To the Chalcidides the species of Megastigmus belong, of which several may be found in different conifer seeds (Pseudotsuga taxifolia Britton, Larix spp., Abies alba Mill, etc.) and further ·Syntomaspis druparum Boh. which infests apple and pear seeds.

In samples of birch seeds gallformed seeds may occur, in which larvae of the birch gallfly, Oligotrophus betulae Winn. are found.

SAPROPHYTIC FUNGI

The infections hitherto discussed are of a parasitic character; some details must be finally added on the effect of saprophytic fungi. Whereas parasitic fungi are mostly characteristic for only one or a few closely related species of seeds, saprophytic fungi are much less specialised and spread on a very great variety of kinds of seeds, especially if the vitality of the latter has suffered for some reason or other. As such saprophytic fungi several species of the following genera may be mentioned: Penicillium (Plate XVIII), Aspergillus (Plate XXXI), Mucor, Rhizopus, Cladosporium (Plate XVII), Alternaria (Plate XXV), Trichothecium (Plate XXX 2), Oedocephalum (Plate XXX 3), Botrytis (Plate XXII), Acrostalagmus, Chaetomium, Sordaria, Acremoniella (= Eidamia) (Plate XXXII 2), Papulaspora (Plate XXXIII), Stemphylium, Stachybotrys, Stysanus (Plate XXXII 1) etc. Some of these fungi may also behave as parasites, e.g. Botrytis cinerea Pers. and Alternaria spp. The fungi, which are the most common on seeds with diminished resistance are Penicillium and, less frequently, Aspergillus. Whereas some saprophytic fungi, such as the two mentioned above, remain local, others are inclined to spread readily in the germination beds. This is, for instance, the case with Rhizopus and Mucor spp. These fungi can entirely overgrow the germination beds so that the normally germinating seeds also become covered by a veil of mycelium without suffering much from it at first. Botrytis cinerea is also a fungus, which spreads readily in the germination beds, though less quickly than Rhizopus and Mucor do. In contradistinction to the two last mentioned fungi, however, Botrytis does much harm to the healthy seedlings. If a centre of Botrytis cinerea infection is found in a germination bed, it will immediately cause decay of the sound rootlets that may be growing in its direction at the point where they come into contact with this mycelium, and will render them soft and greyish coloured. When such diseased seedlings are examined, attention must be paid to the consideration of whether these seedlings actually show the symptoms of disease primarily or whether they have perhaps been healthy and have decayed afterwards in consequence of a secondary infection caused by Botrytis or some other fungus species. Besides Botrytis cinerea, Fusarium sp. also has the property in a high degree of causing healthy seedlings in their neighbourhood to decay quickly.

Acrostalagmus cinnabarinus Corda is chiefly found on somewhat ligneous substrata (f.i. spinach seed). A fungus, which spreads abundantly on the filter paper of the germination beds in consequence of its large number of spores is Botrytis crystallina (Bon.) Sacc. This fungus, forming a brownish covering, with a sandy appearance must be controlled vigorously from the very beginning, as the spores do not require much in the way of germination substratum and will develop abundantly on filter paper, porous porcelain, ground brick stone used for the Hiltner tests, etc. In the seed testing station at Wageningen this fungus has got the popular name of ,,laboratory-fungus'' because of its troublesome qualities. At its first appearance, energetic measures are taken at once to control it, such as disinfection of the germination cupboards by means of formalin. Seeds frequently bearing these spores are various conifer- and Tropaeolum seeds. Except for its very high capacity to spread for which this fungus is very much dreaded in the laboratory, it does not really cause much harm, as it grows only superficially and does not penetrate deeply into the seed coat, or the pericarp, as the case may be, on which it occurs.

In the folowing list the different kinds of seed borne infections, which may be determined on different seeds are mentioned. Among these, several occur which have not been mentioned in the preceding discussion. But, with the help of the methods of testing, herein described, it will not be very difficult to identify also those infections to which particular reference has not been made.

LIST OF SEED-BORNE INFECTIONS
AND INFESTATIONS

Species.	Infections to be determined in part directly from the original dry samples.
Abies alba Mill.	Megastigmus strobilobius Ratz. [1]
Agrostis spp.	Buntkernels. Tilletia decipiens (Pers.) Winter.
Allium spp.	
Alopecurus pratensis L.	Oligotrophus alopecuri E. Reut. [1]
Apium graveolens L.	Septoria apii (Briosi & Cav.) Chester.
Avena elatior L.	Ustilago perennans Rostrup.
Avena sativa L.	Claviceps purpurea (Fr.) Tul. Oscinis frit L. [1] Oscinis pusilla Meig. [1] Botrytis sclerotia. *As to storehouse-pests see Triticum.
Beta vulgaris L.	
Betula spp.	Oligotrophus betulae Winn. [1]

[1] = animal pests.

Infections to be determined by examining the seeds or seedlings in the germination beds.	Infections to be fixed by shaking the seeds in water or other liquids.
Megastigmus strobilobius Ratz. [1]	
	Tilletia decipiens (Pers.) Winter.
Sclerotinia porri v. Beyma. Botrytis allii Munn. Macrosporium porri Ellis.	Urocystis cepulae Frost.
Septoria apii (Briosi & Cav.) Chester. Phoma sp. Macrosporium sp. Alternaria radicina M. & Dr. & E.	
Gibberella Saubinetii (Mont.) Sacc. Fusarium culmorum (W. Sm.) Sacc. Fusarium avenaceum (Fr.) Sacc. Helminthosporium avenae Eidam. Cladosporium herbarum Link. Alternaria spp. Botrytis cinerea Pers. Colletotrichum graminicolum (Ces.) Wilson. Bacterium coronafaciens Elliott. Oscinis frit L. [1] Oscinis pusilla Meig. [1]	Ustilago levis (Kellerm. & Swingle) Magn. Ustilago avenae (Pers.) Jensen.
Phoma betae Frank.	Uromyces betae (Pers.) Tul.

Species.	Infections to be determined in part directly from the original dry samples.
Brassica spp.	Meligethes aeneus Fab. [1] Ceutorrhynchus assimilis Payk. [1]
Cannabis sativa L.	
Chamaecyparis sp.	
Cryptomeria japonica Don.	Megastigmus cryptomeriae Yano. [1]
Dactylis glomerata L.	Aplanobacter Rathayi E.F.S. = Erwinia Rathayi.
Daucus Carota L.	
Gossypium spp.	Platyedra gossypiella Saund. [1]
Hordeum vulgare L.	Claviceps purpurea (Fr.) Tul. Botrytis sclerotia. Anisoplia sp. [1] Oryzaephilus surinamensis L. [1] *As to storehouse-pests see Triticum.
Lactuca sativa L.	

[1] = animal pests.

Infections to be determined by examining the seeds or seedlings in the germination beds.	Infections to be fixed by shaking the seeds in water or other liquids.
Phoma Lingam (Tode) Desm. Phoma oleracea Sacc. Botrytis cinerea Pers. Alternaria brassicae (Berk.) Bolle. Alternaria circinans (Berk. & Curt.) Bolle. Alternaria herculea E. & M. Mycosphaerella brassicola (Fr.) Lindau. Sphaeronema sp.	
Botrytis cinerea Pers. Fusarium sp.	
Pestalotia sp. (= Pestalozzia sp.)	
Megastigmus cryptomeriae Yano. [1]	
Alternaria radicina M. & Dr. & E.	
Platyedra gossypiella Saund. [1]	
Fusarium spp. Gibberella Saubinetii (Mont.) Sacc. Helminthosporium gramineum Rab. Helminthosporium teres Sacc. Helminthosporium sativum P. K. & B. Cladosporium herbarum Link. Botrytis cinerea Pers. Bacterium cerealinum (Gentner) Elliott. Bacterium translucens Jones, Johns. & Red.	Ustilago hordei (Pers.) Kellerm. & Swingle. Ustilago nuda (Jens.) Kellerm. & Swingle. Tilletia pancicii Bub. & Ran.
Fusarium sp. Alternaria sp. Botrytis cinerea Pers.	

Species.	Infections to be determined in part directly from the original dry samples.
Larix leptolepis Gord.	Megastigmus sp. [1] Eurytoma laricis Yano. [1]
Lens esculenta Mnch.	Bruchus lentis Sch. [1]
Linum usitatissimum L.	Thrips linarius Uzel. [1]
Lupinus spp.	
Lycopersicum esculentum Mill.	
Medicago sativa L.	Apion sp. [1] Liposcelis (Troctes) divinatorius Müll. [1]
Medicago lupulina L.	

[1] = animal pests.

Infections to be determined by examining the seeds or seedlings in the germination beds.	Infections to be fixed by shaking the seeds in water or other liquids.
Megastigmus sp. [1]) Eurytoma laricis Yano. [1])	
Bruchus lentis Sch. [1])	
Colletotrichum linicolum Pethybridge & Lafferty. Botrytis cinerea Pers. forma lini v. Beyma. Polyspora lini Pethybridge & Lafferty. Phoma sp. Fusarium lini Bolley.	
Botrytis cinerea Pers. Fusarium sp. Trichothecium roseum Link.	
Didymella lycopersici Kleb. = Diplodina lycopersici Hollos. Phoma destructiva Plowr. Colletotrichum phomoïdes (Sacc.) Chester. Erwinia michiganense E.F.S.	Cladosporium fulvum Cooke.
Botrytis cinerea Pers. Fusarium sp. Macrosporium sarciniforme Cav. = Pleospora sp.	
Phoma sp.	

Species.	Infections to be determined in part directly from the original dry samples.
Oryza sativa L.	Ustilaginoidea virens (Cke.) Tak. Calandra oryzae L. [1] Calandra granaria L. [1] Oryzaephilus surinamensis L. [1] Tenebrioides mauritanicus L. [1] Rhizopertha dominica Fab. [1] Catharthus gemellatus Duv. [1] Tinea granella L. [1]
Papaver spp.	Ceutorrhynchus macula alba Hbst. [1]
Petroselinum sativum Hoffm.	Septoria petroselini Desm.
Phaseolus spp.	Bruchidius obtectus Say. [1] Sclerotinia sclerotiorum (Lib.) Massee.
Pisum spp.	Bruchus pisorum L. [1] Grapholitha nebritana Tr. [1] Grapholitha dorsana Fb. [1] Apion vorax Hbst. [1]

[1] = animal pests.

Infections to be determined by examining the seeds or seedlings in the germination beds.	Infections to be fixed by shaking the seeds in water or other liquids.
Helminthosporium oryzae v. Breda de Haan. Lisea Fujikuroi Sawada. Piricularia oryzae Cav. Pseudomonas oryzae Uyeda & Ishiyama.	
Dendryphium sp.	
Septoria petroselini Desm. Macrosporium sp.	
Colletotrichum Lindemuthianum (Sacc. & Magn.) Briosi & Cav. Ascochyta Boltshauseri Sacc. Macrosporium commune Rab. Fusarium spp. Cladosporium herbarum Link. Bacterium flaccumfaciens Hedges. Pseudomonas medicaginis f. sp. phaseolicola Burk. Bacterium spp. Bruchidius obtectus Say. [1] (Larva).	
Ascochyta pisi Lib. Ascochyta pinodella Jones. Mycosphaerella pinodes (Berk. & Blox) Stone. Fusarium spp. Botrytis cinerea Pers. Macrosporium commune Rab. = Pleospora herbarum (Pers.) Rab. Cladosporium pisicolum Snyder. Sclerotinia sclerotiorum (Lib.) Massee. Peronospora viciae Berk. Bacterium spp. Marsh spot. Bruchus pisorum L. [1] (Larva).	

Species.	Infections to be determined in part directly from the original dry samples.
seudotsuga taxifolia Britton.	Megastigmus spermotrophus Wachtl. [1]
phanus sativus L.	sclerotia from Sclerotinia sclerotiorum (Lib.) Massee.
osa spp.	Megastigmus aculeatus Swed. [1]
orzonera hispanica L.	
cale cereale L.	Claviceps purpurea (Fr.) Tul. Botrytis sclerotia. Anisoplia sp. [1] Zabrus tenebrioides Goeze. [1] Oryzaephilus surinamensis L. [1] Hadena basilinea L. [1] *As to storehouse-pests see Triticum
inacia oleracea L.	
ija occidentalis L.	
olium pratense L.	Typhula trifolii Rostrup. Botrytis sclerotia sclerotia from Sclerotinia trifoliorum Eriks. Apion sp. [1]

= animal pests.

Infections to be determined by examining the seeds or seedlings in the germination beds.	Infections to be fixed by shaking the seeds in water or other liquids.
Megastigmus spermotrophus Wachtl. [1])	
Alternaria spp.	
Megastigmus aculeatus Swed. [1])	
Sclerotinia sclerotiorum (Lib.) Massee. Botrytis cinerea Pers.	
Fusarium nivale (Fr.) Ces. Fusarium spp. Botrytis cinerea Pers.	Urocystis occulta (Wal.) Rab.
Phoma sp. Colletotrichum spinaciae Ell. & Halst. Fusarium spp. Botrytis cinerea Pers.	
Pestalotia sp. (= Pestalozzia sp.).	
Botrytis cinerea Pers. Botrytis anthophila Bond. Botrytis trifolii v. Beyma. Fusarium sp. Macrosporium sarciniforme Cav. = Pleospora sp.	

Species.	Infections to be determined in part directly from the original dry samples.
Trisetum flavescens P.B.	Tylenchus trisetum Kühn.[1]
Triticum vulgare Aschrs. & Gr.	Claviceps purpurea (Fr.) Tul. Tylenchus scandens Schneid (ear-cockles).[1] Botrytis sclerotia. Bunt kernels (Tilletia spp.). *Calandra granaria L.[1] *Tenebrioides mauritanicus L.[1] *Laemophloeus ferrugineus Steph.[1] Anisoplia segetum Hbst.[1] Anisoplia austriaca Hbst.[1] Anisoplia lata Er.[1] Zabrus tenebrioides Goeze.[1] Oryzaephilus surinamensis L.[1] Hadena basilinea F.[1] *Tinea granella L.[1] *Sitotroga cerealella Ol.[1] Contarinia tritici Kirby.[1] *Aleurobius farinae L.[1]
Tsuga Sieboldi Carr.	Callimome tsugae Yano.[1]
Valerianella olitoria Poll.	
Vicia Faba L.	Bruchus atomarius (granarius) L.[1] Bruchus rufimanus Boh.[1]
Zea Mays L.	Ustilago zeae (Beckm.) Ung. Pyrausta nubilalis Hb.[1] Calandra zeae mays Motsch.[1]

[1] = animal pests.

Infections to be determined by examining the seed or seedlings in the germination beds.	Infections to be fixed by shaking the seeds in water or other liquids.
Gibberella Saubinetii (Mont.) Sacc. Fusarium culmorum (W. Sm.) Sacc. Fusarium herbarum (Cda.) Fr. Fusarium avenaceum (Fr.) Sacc. Helminthosporium sativum P.K. & B. Cladosporium herbarum Link. Alternaria spp. Botrytis cinerea Pers.	Tilletia tritici (Bjerkander) Winter. Tilletia levis Kühn. = Tilletia foetens. (Berk & Curtis) Trelease.
Phoma sp.	
Fusarium spp. Ascochyta viciae Lib. Bruchus atomarius L. [1] (Larva). Bruchus rufimanus Boh. [1] (Larva).	
Diplodia maydis (Perk.) Sacc. Fusarium moniliforma Sheldon. Gibberella Saubinetii (Mont.) Sacc. Helminthosporium sp. Sclerospora philippinensis Weston.	Ustilago zeae (Beckm.) Ung.

LITERATURE CITED

1. Alcock, N. L., Seed of Trifolium repens L. carrying a fungus resembling Sclerotinia trifoliorum, clover stem rot. Proceedings of the Intern. Seed Testing Assoc. VI, 1928.
2. Alcock, N. L. & Martin, M. S., A seed-borne disease of clover (Trifolium repens L.). Trans. & Proc. Bot. Soc. Edinburgh. XXX, 1, 1928.
3. Appel, O. & Wollenweber, H. W., Grundlagen einer Monographie der Gattung Fusarium (Link.). Arb. aus der Kaiserl. Biol. Anst. f. Land- und Forstwirtschaft VIII, 1, 1910.
4. Atanasoff, D., The Dilophospora disease of cereals. Phytopathology XV, 1, 1925 & Tijdschrift over Plantenziekten 1924.
5. Beyma thoe Kingma, F. H. v., Über eine Sclerotinia-Art von Porreesamen. Mededeelingen van het Phytopath. Laboratorium „Willie Commelin Scholten", X, 1927.
6. Bondarzew, A. S., Eine neue Krankheit der Blüten des Rotklees, im Zusammenhange mit seiner Fruktifikation (Russian). Bolezni rastenij, St. Petersburg, I, 1914.
7. Bredemann, G., Über die quantitative Bestimmung der Steinbrandsporen im Saatgut nebst Untersuchungen anerkannter Saatweizen auf Brandsporengehalt. Forschungen auf dem Gebiete des Pflanzenbaus und der Pflanzenzüchtung, 1929. (Festschrift zum siebzigsten Geburtstag von Kurt von Rümker.)
8. Clayton, E. E., Black-leg disease of Brussels sprouts, cabbage and cauliflower. New York State Agr. Exp. Sta. Geneva N.Y. Bull. 550, 1927.
9. Cole, Grace, M., A discussion of abnormal sprouts occurring in onion seed germination. Proc. of the 18th Ann. Meeting of the Assoc. of Off. Seed Analysts of North America, 1926.
10. le Cosquino de Bussy, Ivonne J., De bacterieziekte van de boon (Phaseolus vulgaris L.), veroorzaakt door Pseudomonas medicaginis f. sp. phaseolicola Burk. Dissertatie, Utrecht 1936.
11. Crosier, W. F., Patrick, S. R. & Munn, M. T., Abnormal germination resulting from improperly galvanized trays. Phytopathology XXVII, 8, 1937.
12. Crosier, W. F., Patrick, S. R. & Willits, C. O., Zinc injury in germination tests. Proceedings of the Intern. Seed Testing Assoc. IX, 2, 1937.
13. Cunningham, G. H., Dry-rot of Swedes and Turnips: its cause and control. New Zealand Dep. of Agr. Bull. 133, 1927.
14. Dounine, M. S. & Michailow, N. J., Über Bestimmung des Brand-Sporengehaltes an Getreidesamen. Mitt. der Intern. Ver. f. Samenkontrolle VII, 13–17, 1931.
15. Drechsler, Ch., Some graminicolous species of Helminthosporium. Journ. of Agr. Research XXIV, 8, 1923.
16. Gassner, G., Neue Wege zur Bekämpfung des Weizenflugbrandes durch Beizung. Phytopath. Zeitschrift V, 5, 1933.

17. Gassner, G. & Kirchhoff, H., Versuche zur Bekämpfung des Weizenflug-brandes mittels Benetzungsbeize. Phytopath. Zeitschrift VI, 5, 1933.

18. Gassner, G. & Kirchhoff, H., Die Bedeutung der Wasseraufnahme des Weizenkorns, insbesondere des Weizenembryos, für Wirkung und Wirkungsweise der Warmwassertauch- und Benetzungsbeize. Phytopath. Zeitschrift IX, 3, 1936.

19. Gentner, G., Eine Methode zum Nachweis der Sporen des Steinbrandes und anderer Pilzarten am Saatgut. Fortschritte der Landwirtschaft IV, 11, 1929.

20. Goossens, J. A. A. M. H., Onderzoek over de door Phoma apiicola Klebahn veroorzaakte schurftziekte van de Knolselderijplant, Apium graveolens L. en over synergetische vormen en locale rassen van deze zwam. Dissertatie, Wageningen, 1928.

21. (Hiltner-Methode), Technische Vorschriften für die Prüfung von Saatgut, gültig vom 1. Juli 1916 an. Landwirtsch. Versuchs-Stationen LXXXIX, 1917.

22. Hus, P., Onderzoekingen betreffende de vallersziekte bij kool, veroorzaakt door Phoma oleracea. Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst in het jaar 1930, pag. 144, Sept. 1931.

23. Jones, L. K., Studies of the nature and control of blight, leaf- and podspot and footrot of peas, caused by species of Ascochyta. Geneva Agr. Exp. Sta. Bull. 547, 1927.

24. Kitunen, E., Untersuchungen über die Lebensweise des Haferbrandes Ustilago avenae (Persoon) Jensen. Suomen Maataloustieteellisen seuran julkaisuja XXXV, 2, 1937.

25. Löhnis, Marie P., Wat veroorzaakt kwade harten in erwten? Tijdschrift over Plantenziekten XLII, 6, 1936.

26. Munn, M. T., The sanitary condition of Brassica seeds received from various sources. Report of the 8th Internat. Seed Testing Congress in Zürich. Proceedings of the Intern. Seed Testing Assoc., 1938.

27. Neergaard, P., Attacks of Alternaria radicina on Celery and Carrot. Royal veterinary and agricultural college. Yearbook 1937.

28. Oort, A. J. P., Een nieuwe methode ter bestrijding van tarwestuifbrand (Ustilago tritici). Tijdschrift over Plantenziekten, XL, 1934.

29. Pape, H., Beiträge zur Biologie und Bekämpfung des Kleekrebses (Sclerotinia trifoliorum Erikss.). Arb. aus der Biol. Reichsanstalt für Land- und Forstwirtschaft, XXII, 2, 1937.

30. Pethybridge, G. H., Marsh spot in pea seeds: is it a deficiency disease? Journ. Min. Agr. Gt. Britt., XLIII, 1, 1936.

31. Schaffnit, E., Der Schneeschimmel und die übrigen durch Fusarium nivale Ces. hervorgerufenen Krankheitserscheinungen des Getreides. Aus der Abt. für Pflanzenkrankheiten des Kaiser Wilhelms-Instituts für Landwirtschaft in Bromberg, 1932.

32. Schoevers, T. A. C., De overgang van den tomatenkanker met het zaad. Verslagen en Mededeelingen van den Plantenziektenkundigen Dienst, Wageningen, no 56, 1929.

33. Voisenat, P., Quelques observations sur les anomalies de la germination des semences. Académie d'Agriculture de France, XVIII, 1932.

34. Wehlburg, C., Onderzoekingen over erwten-anthracnose. Dissertatie, Utrecht, 1932.
35. Wollenweber, H. W., Fusaria autographice delineata. Annales Mycologici, XV, 1/2, 1917.
36. Wollenweber, H. W. & Reinking, O. A., Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. Paul Parey, Berlin, 1935.
37. Zacher, Fr., Die Vorrat-, Speicher- und Materialschädlinge und ihre Bekämpfung. Paul Parey, Berlin, 1927.
38. Zacher, Fr., Die tierischen Samenschädlinge in Freiland und Lager. Wissenschaft und Technik des Gartenbaues, V, 1932.
39. Zade, A., Neuere Untersuchungen über die Lebensweise und Bekämpfung des Haferflugbrandes, Ustilago Avenae. Angew. Botanik VI, 2, 1924.

CONTENTS